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**Cheese and processed cheese
products — Determination of fat
content — Gravimetric method
(Reference method)**

*Fromage et fromage fondu — Détermination de la teneur en matière
grasse — Méthode gravimétrique (Méthode de référence)*



Reference numbers
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 1735 | IDF 5 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This edition of ISO 1735 | IDF 5 cancels and replaces ISO 1735:1987, which has been technically revised.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

International Standard ISO 1735 | IDF 5 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Fat*, of the Standing Committee, *Main components in milk*, under the aegis of its project leader, Mr G.J. Beutick (NL).

This edition of ISO 1735 | IDF 5 cancels and replaces IDF 5B:1986, which has been technically revised.

Cheese and processed cheese products — Determination of fat content — Gravimetric method (Reference method)

WARNING — The use of ISO 1735 | IDF 5 may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish safety and health practices and to determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies the reference method for the determination of the fat content of all types of cheese and processed cheese products having lactose contents of below 5 % (mass fraction) of non-fat solids.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3889:1977, *Milk and milk products — Determination of fat content — Mojonnier-type fat extraction flasks*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

fat content of cheese and processed cheese products

mass fraction of substances determined by the procedure specified in this International Standard

NOTE The fat content is expressed as a percentage by mass.

4 Principle

A test portion is digested with hydrochloric acid then ethanol is added. The acid-ethanolic solution is extracted with diethyl ether and light petroleum and the solvents are removed by distillation or evaporation. The mass of the substances extracted is determined. This is usually known as the Schmid-Bondzynski-Ratzlaff principle.

5 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity. The reagents shall leave no appreciable residue when the determination is carried out by the method specified (see 5.1).

5.1 Purity of reagents

To test the quality of the reagents, carry out a blank test as specified in 9.2. Use an empty fat-collecting vessel, prepared as specified in 9.3, for mass control purposes.

The reagents shall leave no residue greater than 0,5 mg (see A.1 of Annex A).

If the residue of the complete reagent blank test is greater than 0,5 mg, determine the residue of the solvents separately by distilling 100 ml of the diethyl ether and light petroleum, respectively. Use an empty control vessel to obtain the real mass of residue, which shall not exceed 0,5 mg. Replace unsatisfactory reagents or solvents, or redistil solvents.

Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after the redistillation.

5.2 Concentrated hydrochloric acid, ρ_{20} (HCl) = 1,18 g/ml.

5.3 Dilute hydrochloric acid, ρ_{20} (HCl) = 1,125 g/ml approximately.

Dilute 675 ml of concentrated hydrochloric acid (5.2) to 1 000 ml with water and mix.

5.4 Ethanol, or ethanol denatured by methanol, containing at least 94 % (volume fraction) of ethanol (see A.5).

5.5 Diethyl ether, free from peroxides (see A.4), complying with the requirements for the blank test.

5.6 Light petroleum, with any boiling range between 30 °C and 60 °C or, as equivalent, **pentane** [$\text{CH}_3(\text{CH}_2)_3\text{CH}_3$], with a boiling point of 36 °C.

NOTE The use of pentane is recommended due to its higher purity and constant quality.

5.7 Mixed solvent, prepared shortly before use by mixing equal volumes of diethyl ether (5.5) and light petroleum (5.6).

6 Apparatus

WARNING — Since the determination involves the use of volatile flammable solvents, electrical apparatus employed may be required to comply with legislation relating to the hazards in using such solvents.

Usual laboratory equipment and, in particular, the following.

6.1 Analytical balance, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.

6.2 Centrifuge, capable of holding the fat-extraction flasks or tubes (6.6) and capable of spinning at a rotational frequency of 500 min^{-1} to 600 min^{-1} to produce a radial acceleration of 80 g to 90 g at the outer end of the flasks or tubes.

NOTE The use of the centrifuge is optional but recommended (see 9.4.7).

6.3 Distillation or evaporation apparatus, capable of distilling the solvents and ethanol from the flasks or capable of evaporating from beakers and dishes (9.4.13) at a temperature not exceeding 100 °C.

6.4 Drying oven, electrically heated, with ventilation port(s) fully open, capable of being maintained at a temperature of $102\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ throughout the working space. The oven shall be fitted with a suitable thermometer.

6.5 Boiling water bath or hot plate.

6.6 Fat-extraction flasks, Mojonnier-type, as specified in ISO 3889.

NOTE It is also possible to use fat-extraction tubes, with siphon or wash-bottle fittings, but the procedure is slightly different then. The alternative procedure is specified in Annex B.

The flasks shall be provided with good quality bark corks or stoppers of other material unaffected by the reagents used. Extract bark corks with the diethyl ether (5.5), then place in water at $60\text{ }^{\circ}\text{C}$ or more for at least 15 min. Then allow them to cool in the water so that they are saturated when used.

6.7 Rack, capable of holding the fat-extraction flasks (or tubes) (6.6).

6.8 Wash bottle, suitable for use with the mixed solvent (5.7). Do not use plastic wash bottles.

6.9 Fat-collecting vessels, for example: boiling flasks, flat-bottomed, of capacity 125 ml to 250 ml; conical flasks, of capacity 250 ml; or metal dishes made of stainless steel, flat-bottomed, of diameter 80 mm to 100 mm, of height approximately 50 mm. Do not use aluminium dishes.

6.10 Boiling aids, fat-free, of non-porous porcelain or silicon carbide (optional if using metal dishes).

6.11 Measuring cylinders, of capacities 5 ml and 25 ml.

6.12 Pipettes, graduated, to deliver 10 ml.

6.13 Tongs, made of metal, capable of holding flasks, beakers or dishes.

6.14 Sheets of cellulose film, unlacquered, soluble in hydrochloric acid, of thickness 0,03 mm to 0,05 mm, of dimensions 50 mm \times 75 mm approximately. The sheets shall be inert under the test conditions.

6.15 Appropriate grinding or grating device, easy to clean, for preparing the test sample.

7 Sampling

It is important that the laboratory receive a test sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

The test samples shall be kept at a temperature of between $0\text{ }^{\circ}\text{C}$ and $20\text{ }^{\circ}\text{C}$ from the time of sampling to the time of commencing the procedure.

8 Preparation of test sample

Prior to analysis, remove the rind, the smear or the mouldy surface layer of the cheese in such a way as to obtain a test sample representative of the cheese as it is usually consumed.

Grind or grate the test sample by using an appropriate grinding or grating device (6.15). Mix the ground mass quickly and, if necessary for semi-hard and hard cheeses, grind it a second time and again mix thoroughly. Preferably, cut hard and semi-hard cheeses into cubes of about 15 mm \times 15 mm. Mix the cubes by shaking in a container. Grind or grate the test sample as specified before. Clean the device after preparing each sample.

If the test sample cannot be ground or grated, mix it thoroughly by intensive kneading, for example with a pestle in a mortar. Care should be taken to avoid moisture loss.

Store the test sample in an airtight container until commencing the analysis, which shall be carried out as soon as possible after grinding.

If, however, a delay is unavoidable, take all precautions to ensure proper preservation of the test sample. When refrigerated, bring the test sample to room temperature. Thoroughly mix the sample to obviate the well-documented transfer of moisture within the cheese that occurs during cooling and warming. Ensure that any condensation of moisture on the inside surface of the container is thoroughly and uniformly re-incorporated into the test sample. Do not examine ground cheese showing unwanted mould growth or signs of deterioration.

All sample preparation should be carried out in a manner which minimizes moisture loss. Such moisture loss will have the effect of increasing the apparent fat content.

9 Procedure

NOTE An alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings is specified in Annex B.

9.1 Test portion

Mix the test sample (Clause 8) by gently stirring. Immediately weigh, to the nearest 1 mg, directly or by difference, 1 g to 3 g of test sample into a fat-extraction flask (6.6), a 100 ml beaker or flask.

Weigh an amount of 3 g for cheeses having a mass fraction of fat of up to 30 %. For cheeses having a mass fraction of fat of more than 30 %, adapt the mass of the test portion so as to obtain a mass of extracted fat of between 750 mg and 1 000 mg.

The test portion may also be weighed on a sheet of cellulose film (6.14), which is subsequently folded and introduced into the chosen vessel. Deliver the test portion as completely as possible into the lower (small) bulb of the fat-extraction flask.

9.2 Blank test

Carry out a blank test simultaneously with the determination, using the same procedure and same reagents but omitting the test portion (see A.2).

If the value obtained in the blank test regularly exceeds 1,0 mg, check the reagents if this has not been done recently (see 5.1). Corrections for values of more than 2,5 mg in the blank test shall be reported in the test report.

9.3 Preparation of fat-collecting vessel

Dry a fat-collecting vessel (6.9) with a few boiling aids (6.10) in the drying oven (6.4) set at 102 °C for 1 h.

NOTE Boiling aids are desirable to promote gentle boiling during the subsequent removal of solvent, especially in the case of glass vessels; their use is optional in the case of metal dishes.

Allow the fat-collecting vessel to cool (protected from dust) to the temperature of the weighing room (glass vessel for at least 1 h, metal dish for at least 30 min).

To avoid insufficient cooling or unduly long cooling times, the fat-collecting vessel should not be placed in a desiccator.

Use tongs to place the fat-collecting vessel on the balance to avoid, in particular, temperature variations. Weigh the dish to the nearest 1 mg.

9.4 Determination

9.4.1 Depending on the shape of the extraction apparatus and the size of the test portion, add 8 ml to 10 ml of dilute hydrochloric acid (5.3). Add the hydrochloric acid so as to wash the test portion into the small bulb of the fat-extraction flask (6.6) or onto the bottom of the beaker or flask, and mix.

9.4.2 Heat by gently moving the fat-extraction flask or vessel (to avoid charring) in a boiling water bath or on a hot plate (6.5) or over a flame, until all the particles are entirely dissolved.

NOTE Some Mojonnier-type fat-extraction flasks cannot be heated over a flame.

9.4.3 Allow the fat-extraction flask or vessel to stand for 20 min to 30 min in the boiling water bath (6.5) or keep it gently boiling over the flame or on the hot plate (6.5) for 10 min. Cool under running water.

9.4.4 If the digestion has been carried out in the fat-extraction flask apparatus, add 10 ml of ethanol (5.4). Mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards between the two bulbs while not bringing the liquid too near the neck of the flask. Proceed as in 9.4.5.

Alternatively, if the digestion has been carried out in a vessel other than the fat-extraction flask (6.6), pour the contents of the vessel into a fat-extraction flask. Rinse the vessel successively with 10 ml of ethanol (5.4). Mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards between the two bulbs while not bringing the liquid too near the neck of the flask. Then rinse the vessel with 25 ml of diethyl ether (5.5) and pour the vessel contents into the fat-extraction flask, while rinsing the tip or rim with some additional diethyl ether. Close the fat-extraction flask with a cork saturated with water or with a stopper of other material wetted with water (see 6.6) and shake as described in 9.4.5. Finally rinse the vessel again with 25 ml of light petroleum (5.6) and pour that solvent into the fat-extraction flask, while also rinsing the tip or rim with some additional light petroleum. Close the fat-extraction flask again and shake its contents as described in 9.4.6. Then continue with the centrifugation procedure as in 9.4.7.

9.4.5 Add 25 ml of diethyl ether (5.5). Close the fat-extraction flask with a cork saturated with water or with a stopper of other material wetted with water (see 6.6).

Shake the flask vigorously for 1 min, but not so vigorously as to cause formation of a persistent emulsion. While shaking, keep the flask in a horizontal position with the small bulb extending upwards, periodically allowing the liquid in the large bulb to run into the small bulb. If necessary, cool the flask under running water.

Carefully remove the cork or stopper and rinse it and the neck of the flask with a little mixed solvent (5.7). Use the wash bottle (6.8) so that the rinsings run into the fat-extraction flask.

9.4.6 Add 25 ml of the light petroleum (5.6). Close the fat-extraction flask with the rewetted cork or rewetted stopper (by dipping in water).

Gently shake the flask as described in 9.4.5, but for 30 s only.

9.4.7 Centrifuge the closed fat-extraction flask at a rotational frequency of 500 min⁻¹ to 600 min⁻¹ for 1 min to 5 min. If a centrifuge is not available, allow the closed flask to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the fat-extraction flask under running water.

9.4.8 Carefully remove the cork or stopper and rinse it and the inside of the neck of the fat-extraction flask with a little mixed solvent (5.7). Use the wash bottle (6.8) so that the rinsings run into the flask.

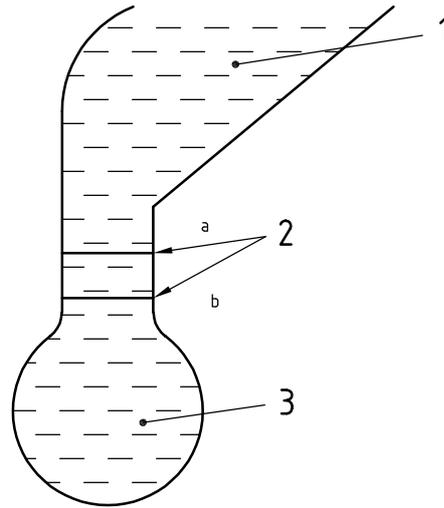
If the interface is below the bottom of the stem of the flask, raise it slightly above this level by gently adding water down the side of the flask (see Figure 1) to facilitate the decanting of solvent.

NOTE In Figures 1 and 2, one of the three types of fat-extraction flasks (6.6) specified in ISO 3889 has been chosen, but this does not imply any preference over other types.

9.4.9 Hold the fat-extraction flask by the small bulb and carefully decant as much as possible of the supernatant layer into the prepared fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) (optional with metal dishes). Avoid decanting any of the aqueous layer (see Figure 2).

9.4.10 Rinse the outside of the neck of the fat-extraction flask with a little mixed solvent (5.7). Collect the rinsings in the fat-collecting vessel; take care that the mixed solvent does not spread over the outside of the extraction flask. If desired, remove the solvent or a part of it from the fat-collecting vessel by distillation or evaporation as described in 9.4.13.

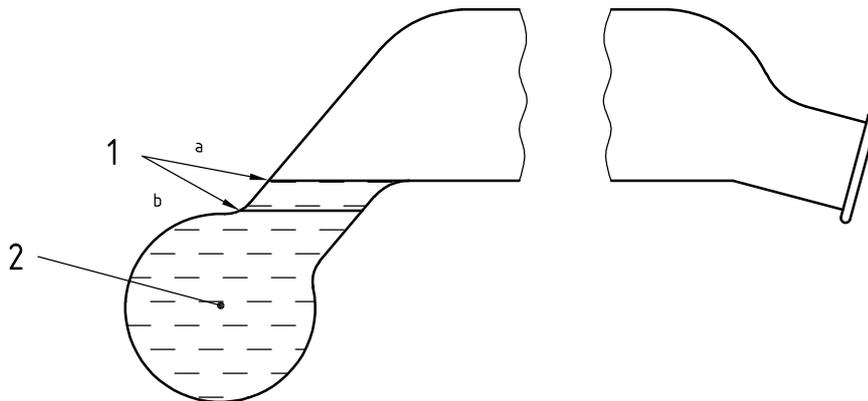
9.4.11 Carry out a second extraction by repeating the operations described in 9.4.5 to 9.4.9 inclusive, using instead of 25 ml only 15 ml of diethyl ether (5.5) and 15 ml of light petroleum (5.6). Use the ether to rinse the inside of the neck of the fat-extraction flask too. If necessary, raise the interface to the middle of the stem of the fat-extraction flask (see Figure 1) to enable the decanting of solvent to be as complete as possible (see Figure 2).



Key

- 1 solvent
- 2 interface
- 3 aqueous layer
- a At second and third extraction.
- b At first extraction.

Figure 1 — Before decanting



Key

- 1 interface
- 2 aqueous layer
- a At second and third extraction.
- b At first extraction.

Figure 2 — After decanting

9.4.12 Carry out a third extraction by again repeating the operations described in 9.4.5 to 9.4.9 inclusive. Again, use only 15 ml of diethyl ether (5.5) and 15 ml of light petroleum (5.6). Use the ether to rinse again the inside of the neck of the fat-extraction flask. If necessary, raise the interface to the middle of the stem of the fat-extraction flask (see Figure 1) to enable the final decantation of solvent to be as complete as possible (see Figure 2).

The third extraction may be omitted for products with a mass fraction of fat of less than 3 %.

9.4.13 Remove the solvents (including the ethanol) as completely as possible from the fat-collection flask by distillation, or from the beaker or dish (6.3) by evaporation. Rinse the inside of the neck of the flask with a little mixed solvent (5.7) before commencing the distillation.

9.4.14 Heat the fat-collecting vessel in the drying oven (6.4) set at 102 °C for 1 h. Remove the fat-collecting vessel from the oven and immediately verify whether or not the fat is clear. If the fat is not clear, fat-extraneous matter is presumed to be present and the whole procedure shall be repeated. If the fat is clear, protect the fat-collecting vessel from dust and allow the vessel to cool to the temperature of the weighing room (glass vessel for at least 1 h, metal dish for at least 30 min).

NOTE Depending on the fat-collecting vessel used, placing it on its side in the drying oven might allow the solvent vapour to escape better.

Do not wipe the fat-collecting vessel immediately before weighing. Use tongs to place the fat-collecting vessel on the balance to avoid, in particular, temperature variations. Weigh to the nearest 1 mg.

9.4.15 Heat the fat-collecting vessel in the drying oven (6.4) set at 102 °C for 30 min. Reweigh and record as described in 9.4.14. Repeat the heating and weighing procedure until the mass of the fat-collecting vessel decreases by 1,0 mg or less, or increases between two successive weighings. Record the minimum mass as the mass of the fat-collecting vessel and extracted matter.

10 Calculation and expression of results

10.1 Calculation

Calculate the mass fraction of fat, w , of the sample using the following equation:

$$w = \frac{(m_1 - m_2) - (m_3 - m_4)}{m_0} \times 100 \%$$

where

m_0 is the numerical value of the mass of the test portion (9.1), in grams;

m_1 is the numerical value of the mass of the fat-collecting vessel and extracted matter in it (9.4.15), in grams;

m_2 is the numerical value of the mass of the prepared fat-collecting vessel (9.3), in grams;

m_3 is the numerical value of the mass of the fat-collecting vessel used in the blank test (9.2) and any extracted matter in it (9.4.15), in grams;

m_4 is the numerical value of the mass of the fat-collecting vessel (9.3) used in the blank test (9.2), in grams.

10.2 Expression of results

Express the results to two decimal places.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are given in Annex C. The values derived from this test may not be applicable to concentration ranges and matrices other than those given.

Laboratories that make use of this method should note that for certain types of cheese higher values for r and R might be found in practice.

11.2 Repeatability

The absolute difference between two individual single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than 0,30 %.

11.3 Reproducibility

The absolute differences between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 0,40 %.

12 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this International Standard;
- d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- e) the test result(s) obtained and, if the repeatability has been checked, the final quoted result obtained.

Annex A (normative)

Additional procedures

A.1 Blank test to check the reagents (see 5.1)

A.1.1 In this blank test, a fat-collecting vessel (6.9) for mass control purposes shall be used in order that changes in the atmospheric condition of the balance room or temperature effects on the fat-collecting vessel will not falsely suggest the presence or absence of non-volatile matter in the extract of the reagents. The control vessel may be used as a counterweight vessel in the case of a two-pan balance. Otherwise, deviations of the apparent mass $[(m_3 - m_4)$ in 10.1] of the control vessel shall be considered when checking the mass of the fat-collecting vessel used for the blank test. Hence, the change in apparent mass of the blank test vessel, corrected for the apparent change in mass of the fat-collecting vessel for control purposes, shall show no increase in mass greater than 1,0 mg.

A.1.2 Very occasionally, the solvents may contain volatile matter which is strongly retained in fat. If there are indications of the presence of such substances, carry out blank tests on all the reagents and for each solvent using a fat-collecting vessel with about 1 g of anhydrous butterfat. If necessary, redistil solvents in the presence of 1 g of anhydrous butterfat per 100 ml of solvent. Use the solvents only shortly after redistillation.

A.2 Blank test carried out simultaneously with the determination (9.2)

The value obtained in the blank test carried out simultaneously with the determination, enables the apparent mass of substances extracted from a test portion ($m_1 - m_2$) to be corrected for the presence of any non-volatile matter derived from the reagents. But also corrections are needed for any change of atmospheric conditions in the balance room and some temperature difference between the fat-collecting vessel (6.9) and the balance room at the two weighings (9.4.15 and 9.3).

Under favourable conditions (low value in the blank test on reagents, stable temperature of the balance room, sufficient cooling time for fat vessel), the value will usually be less than 1,0 mg and may then be neglected in the calculation in the case of routine determinations. Slightly higher values (positive and negative) up to 2,5 mg are also often encountered. After correction for these values, the results will still be accurate. When corrections for a value of more than 2,5 mg are applied, it shall be mentioned in the test report (Clause 12).

If the value obtained in this blank test regularly exceeds 1,0 mg, the reagents should be checked, if no recent check has been made. Any impure reagent shall be replaced or purified (see 5.1 and A.1).

A.3 Test for peroxides

To test for peroxides, add 1 ml of a freshly prepared 100 g/l of potassium iodide solution to 10 ml of diethyl ether (5.5) in a small glass-stoppered cylinder which has been previously rinsed with the ether. Shake the cylinder and allow to stand for 1 min. No yellow colour should be observed in either layer.

Other suitable methods of testing for peroxides may be used.

To ensure that the diethyl ether is free, and is maintained free, from peroxides, treat the ether at least 3 days before it is to be used, as follows.

Cut zinc foil into strips that will reach at least half-way up the bottle containing the diethyl ether, using approximately 80 cm² foil per litre of diethyl ether.

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Before use, completely immerse the strips of foil for 1 min in a solution containing 10 g of copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 2 ml of concentrated [98 % (mass fraction)] sulfuric acid per litre.

Wash the strips gently but thoroughly with water, place the wet copper-plated strips in the bottle containing the diethyl ether, and leave the strips in the bottle.

Other methods may be used provided that they do not affect the result of the determination.

A.4 Diethyl ether containing antioxidants

Diethyl ether containing about 1 mg/kg of antioxidants is available in some countries, especially for fat determinations. This content does not exclude its use for reference purposes.

In other countries, diethyl ether with higher antioxidant contents, for example up to 7 mg/kg, is available. Such diethyl ether should only be used for routine determinations with an obligatory blank test carried out simultaneously with the determination(s) to correct for systematic errors due to the antioxidant residue. For reference purposes, such diethyl ether shall always be distilled before use.

A.5 Ethanol

Ethanol denatured otherwise than by the addition of methanol may be used provided that the denaturant does not affect the result of the determination.

Annex B (informative)

Alternative procedure using fat-extraction tubes with siphon or wash-bottle fittings

B.1 General

If fat-extraction tubes (6.6) with siphon or wash-bottle fittings (see Figure B.1 for an example) are to be used instead of fat-extraction flasks (6.6), use the procedure specified in this annex. The tubes shall be provided with good quality cork stoppers as specified for the fat-extraction flasks (6.6).

B.2 Procedure

B.2.1 Preparation of test sample

See Clause 8.

B.2.2 Test portion

Proceed as specified in 9.1 but using the fat-extraction tubes (6.6).

The test portion shall be delivered as completely as possible to the bottom of the fat-extraction tube.

B.2.3 Blank test

See 9.2 and A.2.

B.2.4 Preparation of fat-collecting vessel

See 9.3.

B.2.5 Determination

B.2.5.1 Carry out the determination without delay.

Add 10 ml of dilute hydrochloric acid (5.3) so as to wash the test portion (B.2.2) onto the bottom of the fat-extraction tube, beaker or flask, and mix.

B.2.5.2 Heat by gently moving the fat-extraction tube or vessel (to avoid charring) in a boiling water bath or on a hot plate (6.5) or over a flame, until all the particles are entirely dissolved.

B.2.5.3 Allow the fat-extraction tube or vessel to stand for 20 min to 30 min in the boiling water bath or on the hot plate or keep it gently boiling over the flame for 10 min. Cool under running water.

B.2.5.4 If the digestion has been carried out in the fat-extraction flask apparatus, add 10 ml of ethanol (5.4). Mix gently but thoroughly by allowing the contents of the flask to flow backwards and forwards between the two bulbs, while not bringing the liquid too near the neck of the flask. Continue as in B.2.5.5.

If, alternatively, the digestion has been carried out in a vessel other than the fat-extraction flask (6.6), pour the contents of the vessel into a fat-extraction flask. Rinse the vessel successively with 10 ml of ethanol (5.4). Mix

gently but thoroughly by allowing the contents of the flask to flow backwards and forwards between the two bulbs, while not bringing the liquid too near the neck of the flask. Then rinse the vessel with 25 ml of diethyl ether (5.5) and pour the vessel contents into the fat-extraction flask, while rinsing the tip or rim with some additional diethyl ether. Close the fat-extraction flask with a cork saturated with water or with a stopper of another material wetted with water (see 6.6) and shake as described in B.2.5.5. Finally rinse the vessel again with 25 ml of light petroleum (5.6) and pour that solvent into the fat-extraction flask, while also rinsing the tip or rim with some additional light petroleum. Close the fat-extraction flask again and shake its contents again as described in B.2.5.6. Continue with the centrifugation procedure as in B.2.5.7.

B.2.5.5 Add 25 ml of diethyl ether (5.5). Close the fat-extraction flask with a cork saturated with water or with a stopper of another material wetted with water (see 6.6).

Shake the flask vigorously for 1 min, but not so vigorously as to cause formation of a persistent emulsion. While shaking, keep the flask in a horizontal position with the small bulb extending upwards, periodically allowing the liquid in the large bulb to run into the small bulb. If necessary, cool the flask under running water.

Carefully remove the cork or stopper and rinse it and the neck of the flask with a little mixed solvent (5.7). Use the wash bottle (6.8) so that the rinsings run into the fat-extraction flask.

B.2.5.6 Add 25 ml of the light petroleum (5.6). Close the fat-extraction flask with the rewetted cork or rewetted stopper (by dipping in water).

Gently shake the flask as described in B.2.5.5, but for 30 s only.

B.2.5.7 Centrifuge the closed tube at a rotational frequency of 500 min⁻¹ to 600 min⁻¹ for between 1 min and 5 min. If a centrifuge is not available, allow the closed tube to stand in the rack (6.7) for at least 30 min until the supernatant layer is clear and distinctly separated from the aqueous layer. If necessary, cool the tube under running water.

B.2.5.8 Carefully remove the cork or stopper and rinse it and the neck of the tube with a little mixed solvent (5.7). Use the wash bottle (6.8) so that the rinsings run into the tube.

B.2.5.9 Insert a siphon or a wash-bottle fitting into the tube. Push down the long inner limb of the fitting until the inlet is approximately 4 mm above the interface between the layers. The inner limb of the fitting shall be parallel to the axis of the extraction tube.

Carefully transfer the supernatant layer out of the tube into the fat-collecting vessel (see 9.3) containing a few boiling aids (6.10) in the case of flasks (optional with metal dishes). Avoid transfer of any of the aqueous layer. Rinse the outlet of the fitting with a little mixed solvent, collecting the rinsings in the fat-collecting vessel.

B.2.5.10 Loosen the fitting from the neck of the tube. Slightly raise the fitting and rinse the lower part of its long inner limb with a little mixed solvent (5.7). Lower and re-insert the fitting and transfer the rinsings to the fat-collecting vessel.

Rinse the outlet of the fitting with a little mixed solvent again, collecting the rinsings in the vessel. If desired, remove the solvent or a part of it from the vessel by distillation or evaporation as described in 9.4.13.

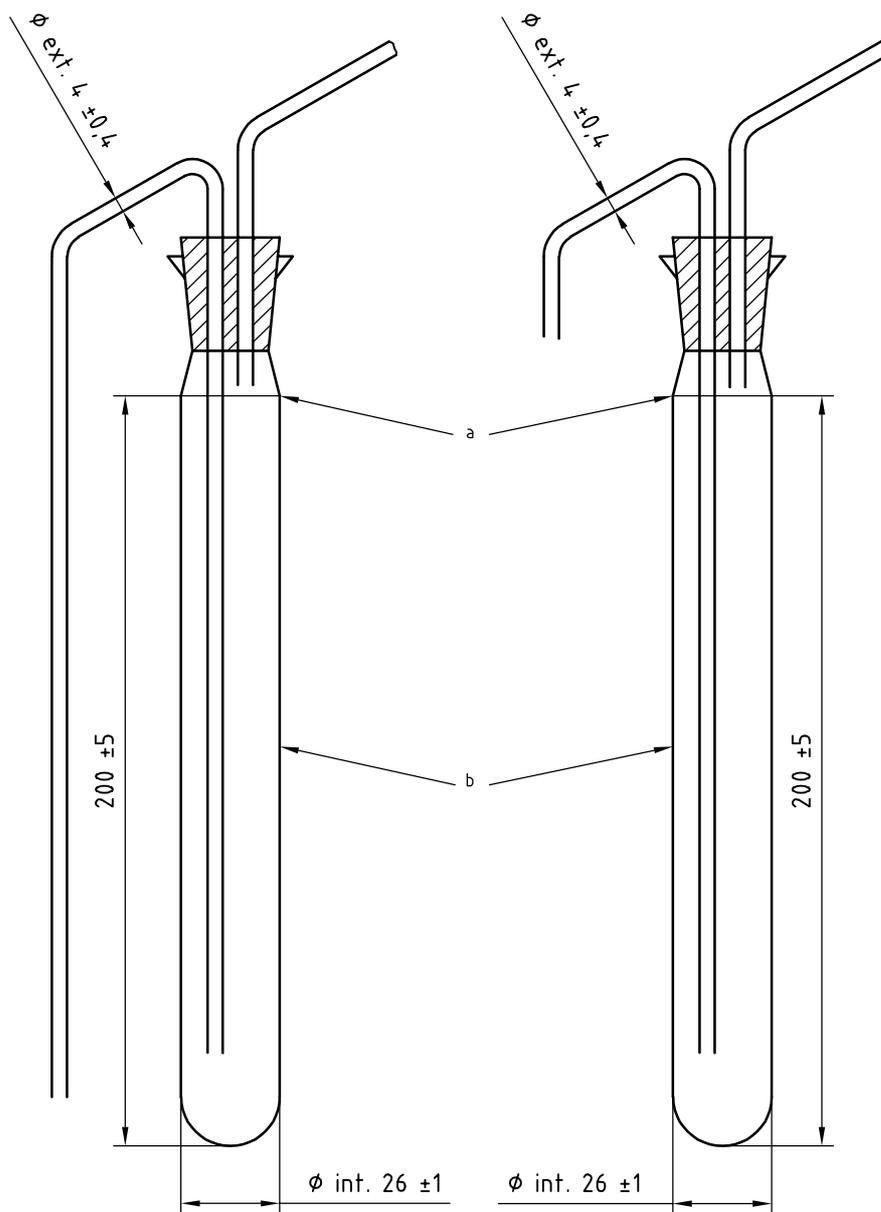
B.2.5.11 Carry out a second extraction by repeating the operations described in B.2.5.5 to B.2.5.10, but instead of 25 ml use only 15 ml of diethyl ether (5.5) and 15 ml of light petroleum (5.6). Use the ether to rinse the longer inner limb of the fitting during the removal of the fitting from the tube after the previous extraction.

B.2.5.12 Carry out a third extraction by again repeating the operations described in B.2.5.5 to B.2.5.10. Again, use only 15 ml of diethyl ether (5.5) and 15 ml of light petroleum (5.6) to rinse the long inner limb of the fitting as described in B.2.5.10.

The third extraction may be omitted for products with a mass fraction of fat of less than 3 %.

B.2.5.13 Proceed as described in 9.4.13 to 9.4.15.

Dimensions in millimetres



a) With siphon fitting

b) With wash-bottle fitting

- a Capacity to this level with fittings removed, $105 \text{ ml} \pm 5 \text{ ml}$.
- b Wall thickness $1,5 \text{ mm} \pm 0,5 \text{ mm}$.

Figure B.1 — Examples of fat-extraction tubes

Annex C (informative)

Interlaboratory test

An international collaborative test involving 12 laboratories from six countries was carried out on two different samples of four types of cheese. The thus-obtained eight test samples were divided again into 16 blind duplicated samples. The test was organized by COKZ (NL). All values are expressed as mass fractions.

The test results were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to give the precision data shown in Table C.1.

Table C.1 — Results of interlaboratory test

	Cheese 1 ^a		Cheese 2 ^b		Cheese 3 ^c		Cheese 4 ^d	
	A	B	A	B	A	B	A	B
Laboratories remaining after eliminating outliers	12	12	12	11	11	11	12	11
Mean value, %	27,12	26,99	29,98	30,69	14,54	9,64	33,01	31,50
Repeatability standard deviation, s_r , %	0,117	0,062	0,116	0,070	0,044	0,039	0,102	0,125
Coefficient of variation of repeatability, %	0,43	0,23	0,39	0,23	0,30	0,40	0,31	0,40
Repeatability limit, r ($= 2,8 s_r$), %	0,332	0,174	0,328	0,197	0,124	0,110	0,290	0,354
Reproducibility standard deviation, s_R , %	0,169	0,096	0,124	0,106	0,081	0,065	0,166	0,210
Coefficient of variation of reproducibility, %	0,62	0,36	0,41	0,35	0,56	0,67	0,50	0,67
Reproducibility limit, R ($= 2,8 s_R$), %	0,479	0,273	0,352	0,299	0,228	0,187	0,470	0,593
^a Emmentaler cheese. ^b Gouda cheese. ^c Processed cheese. ^d Brie cheese.								

Bibliography

- [1] ISO 707¹⁾, *Milk and milk products — Guidance on sampling*
- [2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

1) Equivalent to IDF 50.

ISO 1735:2004(E)
IDF 5:2004(E)

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